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Remarks

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SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	29157		
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Date	November 6, 2006	Reg. No.	30,142

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Marie Secretin
Appl. No.: 10/564,599
Conf. No.: Unknown
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SUBMISSION OF PRIORITY DOCUMENT

Please enter of record in the file of the above application, the attached certified copy of European Patent Application No. 03014055.2 filed on June 23, 2003. Applicant claims priority of June 23, 2003, the earliest filing date of the attached European application under the provisions of Rule 55 and 35 U.S.C. §119, and referred to in the Declaration of this application.

The Commissioner is authorized to charge any fees which may be required, or to credit any overpayment to account No. 02-1818.

Respectfully submitted,

BELL, BOYD & LLOYD LLC

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Robert M. Barrett
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Dated: November 6, 2006

In re Patent Application of: Marie Secretin
INFANT OR FOLLOW-ON FORMULA
Docket No. 112701-702; USSN: 10/564,599
On the date stamped hereon the U.S. Patent and Trademark Office hereby acknowledges receipt of the following:

1. Transmittal Letter (duplicate);
2. Submission of Priority Document (1 pg.); and
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Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. Patent application No. Demande de brevet n°

03014055.2

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 03014055.2
Demande no:

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Anmelder/Applicant(s)/Demandeur(s):

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SUISSE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Nutritional composition

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NO 7539/GF

Patent Application
In the name of Nestec S.A.

Title: Nutritional composition

Nutritional composition

Field of the invention

The present invention relates to a nutritional composition intended for infants and/or young children, as well as to methods for improving gastrointestinal comfort, developing a healthy gut microflora, and promoting the physical development of infants and/or young children by partly or fully feeding said infants or children with the aforementionned nutritional composition.

Background of the invention

The composition of human milk serves as a valuable reference for improving infant formula. However, human milk contains living cells, hormones, active enzymes, immunoglobulins and components with unique molecular structures that cannot be replicated in infant formula. Unlike human milk, infant formula must remain stable on the shelf for up to thirty-six (36) months. These fundamental differences between human milk and infant formula often mandate differences in the composition to achieve similar clinical outcome.

The study of human milk components has stimulated many investigations into what constituents may be added to an improved infant formula. Greater knowledge of the composition of human milk affords the opportunity to design infant formulas that are closer in composition to human milk. However, it becomes increasingly apparent that infant formula can never exactly duplicate human milk. Many constituents in human milk are bioactive and because of synergies among these components, there is little reason to believe that the same compound would have the same bioactivity in infant formula. The likelihood of this possibility is further diminished when the impact of heat treatment for sterilization and long-term storage of the formula is considered.

The composition of human milk differs appreciably from that of other species and much attention has been paid to the various components. Several investigators have reported on the nucleotide content of milk from humans. Numerous publications have also discussed various lipid, oil or fat blends for use in an artificial nutritional for human infants.

There is a need for new formulae, providing to the infant or the young child a nutritional contribution with a unique combination of protective nutrients, especially ensuring growth and metabolic patterns similar to those of breastfed infants, thus resulting in similar health characteristics in later childhood and adulthood.

Summary of the Invention

The present invention therefore pertains to formulae intended both for infants and young children. The formula of the invention comprises proteins, preferably modified sweet whey proteins free or almost free of CGMP, and at least one probiotic strain.

A second object of the present invention is a method for promoting physical development of an infant or a young child consisting in fully or partly feeding said infant or child with the afore-mentioned formula.

A third object of the present invention pertains to a method for improving gastro-intestinal comfort of an infant or a young child consisting in fully or partly feeding said infant or child with the afore-mentioned formula.

A fourth object of the invention relates to a method for developing a healthy gut microflora in an infant or a young child consisting in fully or partly feeding said infant or child with the afore-mentioned formula.

Figures

Figure 1 represents the weight gain of infants from birth to 120 days.
Figure 2 represents the length gain of infants from birth to 120 days.
Figure 3 represents the evolution of the body mass index of infants from birth to 120 days.

Detailed Description of the Invention

In the present specification, the following words are given a definition that must be taken into account when reading and interpreting the description, examples and claims.

Infant: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(a), the term "infants" means children under the age of 12 months. This definition is adopted in the present specification.

Young Children: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(b), the term "young children" means children aged between one and three years. This definition is adopted in the present specification.

Infant formulae: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(c), the term "infant formula" means foodstuffs intended for particular nutritional use by infants during the first four to six months of life and satisfying by themselves the nutritional requirements of this category of persons. This definition is adopted in the present specification. It has to be understood that infants can be fed solely with infant formulas, or that the infant formula can be used by the carer as a complement of human milk. It is synonymous to the widely used expression "starter formula".

Follow-on formulae: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(d), the term "follow-on formulae" means foodstuffs intended for particular nutritional use by infants aged over four months and constituting the principal liquid element in a progressively diversified diet of this category of persons. This definition is adopted in the present specification.

According to a first object of the invention, there is provided a nutritional composition for infants (including a starter composition) or young children. This composition, as already mentionned, is a unique combination of protective nutrients ensuring growth and metabolic patterns similar to those of breastfed infants, thus possibly resulting in similar health characteristics in later childhood and adulthood, characterised by a reduced load on immature organs; although not wishing to be bound by theory, we believe it is especially due to both a source of proteins providing a high protein efficiency, and to a formulation having a low content in electrolytes.
The unique combination of the invention also results in favoring the natural growth of Bifidobacteria in the large intestine as in breastfed infants, in particular due to a high lactose content, a low phosphate and an adapted protein content.

Furthermore, the nutritional composition comprises at least one probiotic selected to exert its beneficial effects all along the intestinal tract and support a healthy gut flora.

Of course, the composition of the formula according to the invention also supplies the infant or the young child with vitamins and minerals recognised as essential for a healthy development, as well as semi-essential nutrients which may be needed in particular conditions. These semi-essential nutrients can include taurine, nucleotides, carnitine, and/or selenium.

The formulas consist of proteins, carbohydrates, probiotics, fats with vitamins and minerals in amounts necessary to provide the sole source of nutrition for healthy term infants from birth until the age of 4-6 months for infant formulas, and as the principal liquid element in a progressively diversified diet of infants aged over four months.

Dietary protein provides the essential amino acids necessary for protein synthesis and growth and protein quality is as important as protein quantity.
Until now, in order to supply enough of the essential amino acids, cow's milk-based infant formulae needed a protein content significantly higher than that of the reference human milk. However, if the amino acid pattern of a cow's milk-based infant formula is closer to that of human milk, the protein content of such a formula can be lowered to resemble that of the reference. A new protein mixture of unique amino acid composition allowing the adaptation of the quantity of protein to a level closer to the average content of human milk has been developed according to an aspect of the present invention.

The protein content of regular whey-adapted formulae ranges from 2.1 to 2.6 g per 100 kcal, whereas the content of human milk ranges from 1.4 to 1.8 g per 100 kcal. Excess protein intake may induce metabolic stress on infant organs that have not fully developed.
Following paediatric recommendations for lowering protein density of infant formulae, clinical trials in infants fed formulae containing protein densities between 1.6 and 2.0 g / 100 kcal have been reported. However, these attempts to lower protein content in a formula using traditional cow's milk protein sources or mixing the currently available fractions – casein and whey -, although demonstrating the principle was conceivable, failed to reproduce all the indices of human milk protein metabolism or to ensure the satisfactory growth of infants.
For instance, results have shown a global plasma amino acid pattern different to that of breast-fed infants, depressed plasma tryptophan levels, elevated plasma threonine levels, delay in growth, and higher energy intake suggesting an increased fat deposition which may be responsible for obesity in later life.

Cow's milk "whey protein" is a mixture of several proteins, which all have a different amino acid profile, and of the non-protein nitrogen (NPN) fraction. The caseino-glyco-macropeptide (CGMP) is a protein fraction that is found in this fraction. It comes from the kappa-casein that is split up by proteolytic cleavage into 2/3 para-kappa-casein, an insoluble fraction that remains in the casein fraction and 1/3 CGMP, a soluble fraction that is found in the whey fraction.

An original fractionation process of whey proteins has been developped and is explained in EP 880902; this process allows the removal of practically all the caseino-glyco-macropeptide (a fraction rich in threonine and poor in tryptophan) from bovine whey thereby increasing the alpha-lactalbumin proportion (a fraction very rich in tryptophan).

By combining this modified sweet whey fraction with skim milk, and with the addition of some free L-histidine and L-arginine (in order to reach the minimum amounts of these amino acids required by EC Directive), the formulation according to the invention has an amino acid profile much closer to that of human milk, characterised in particular by comparable tryptophan and threonine levels, allowing the adaptation of its protein content to that of human milk.

Nutritional value of the new protein mixture used in the manufacture of the protein profile according to the invention has been measured in rats.

The results show (see table 1) that this formulation has a Protein Efficiency Ratio (PER), a nitrogen digestibility, a Biological Value (BV), and a Net Protein Utilisation (NPU) comparable to standard whey-adapted formulae.

Table 1

Nutritional parameters	Casein	standard whey-adapted formula	formulation of the invention
PER	1.36	2.49	2.70
Relative PER (casein = 100%)	100.0	182.8	198.3
Digestibility (%)	96.7	92.8	91.4
BV	0.88	0.96	0.96
NPU (%)	85.4	88.8	87.5

Moreover, rats fed on the formula according to the invention showed significant lower plasma threonine levels and increased plasma tryptophan levels, compared to rats fed on standard whey-adapted formulae.

The protein profile of the composition according to the present invention, with high protein efficiency, is a very well adapted infant formula with a protein content closer to that of human milk. With a protein content being at maximum of 2g/100 kcal, preferably 1.85, most preferably between 1.8 and 1.85 g/100 kcal, it is in the lower part of the range of the most recent paediatric recommendations for infant formulae. Moreover, this level is in line with recent data assessing protein requirements during early life, which has shown that recommendations for optimal protein intakes are lower than they have been reported in the past.

To ensure optimal protein synthesis, and therefore optimal growth, essential and semi-essential (i.e. essential only during infancy) amino acids need to be supplied in the same quantities as in human milk.

The formulation according to the invention is preferably either whey enriched (casein / whey ratio set around 40/60 or lower, such as 45/55), either whey predominant (casein / whey ratio preferably set at 30/70 or even more, such as 20/80). Together with the with the unique protein mixture and casein / whey ratio, the amino acid profile of the composition according to the invention is comparable to that of human milk (see table 2).

Table 2

Amino acid (g / 16 g N)	Human milk			Invention (representative values)
	mean	lowest value	highest value	
Isoleucine *	6.4	5.7	6.8	5.8
Leucine *	11.5	11.0	11.9	11.9
Lysine *	7.9	7.4	8.4	10.0
Methionine *	1.7	1.3	2.1	2.5
Cystine **	2.3	1.7	2.9	2.4
Phenylalanine*	4.6	4.2	5.1	4.6
Tyrosine **	4.7	3.3	6.3	4.0
Threonine *	5.6	5.3	6.6	5.4
Tryptophan *	2.3	1.8	2.6	2.1
Valine *	6.8	5.9	8.0	5.9
Arginine **	4.2	3.5	4.9	4.5
Histidine **	2.8	2.4	3.8	2.5
Alanine	4.8	4.5	5.3	5.1
Aspartic acid	10.4	10.1	10.8	11.1
Glutamic acid	19.6	17.6	22.7	19.7
Glycine	3.2	2.8	3.6	2.7
Proline	10.2	8.9	11.2	7.8

Serine	5.6	5.0	5.9	5.3

All values corrected to 40% NH_3
* essential amino acids ** semi-essential amino acids

Preferably, the proteins are non-hydrolysed proteins.

The sole source of carbohydrates of the composition according to the present invention is lactose. In less preferred embodiments of the invention, however, other sources of carbohydrates, such as for example saccharose, maltodextrins, and/or starch can be used together with lactose, in various ratios.

Carbohydrates constitute an important source of energy in the diet of the newborn infant. Lactose is the natural carbohydrate in human milk. Most infants in good health can digest lactose adequately.

Lactose is associated with stool acidity and an intestinal flora (preponderance of lactobacilli and bifidobacteria) which may be important in suppressing the growth of undesirable bacteria in the intestine of breast-fed infants. Moreover, lactose has been shown to enhance absorption and retention of calcium and probably other minerals. In a recent study, it has been shown that calcium absorption is 10% greater from a lactose-containing formula compared with the same formula in which the lactose was replaced by glucose polymers.

According to one aspect of the invention, the infant formula comprises at least one probiotic, in order to offer all infants, whatever their mode of delivery or their hygienic environment, the advantages of a protective intestinal flora.

A preferred probiotic consist in Bifidobacteria, which as a whole are safe and are L (+) lactic acid producing cultures. A particularly preferred probiotic is *Bifidobacterium lactis*, first sold by Christian Hansen company. *B.lactis* is a Gram-positive, catalase negative strain , producing only L(+) lactic acid. The strain *Bifidobacterium lactis* (BL) has been selected to be added to the present formulae mostly because of its resistance towards acid and bile salts, and its survival not only in products with a short shelf life such as chilled dairy products, but also in powder milks with a longer shelf life such as infant formulae.

B. lactis appearance in the faeces of adults during ingestion of the probiotic has been confirmed by a specific PCR-ELISA method. In infants, 2 studies have been looking to

the presence of B. lactis in the stools of infants fed a formula enriched with B. lactis, either by classical microscopical examination or by the more specific method of Random Amplified Polymorphic DNA (RAPD)-PCR method. It was possible to estimate the percentage of recovery of B. lactis in the stools to be in the range of 25-30%, a value that has been found for other strains of bifidobacteria and is significantly higher than other strains of probiotics

Another preferred probiotic consists in a Streptococcus, particularly *Streptococcus thermophilus* provided under the name TH4 by Chr. Hansen, Denmark. Both *Bifidobaterium lactis* and *Streptococcus thermophilus* have been given a GRAS status (generally recognised as safe) by the USFDA (United Staes Food and Drug Administration) for use in formulas intended for children over 4 months of age, and the same USFDA has authorized marketing of a starter formula enriched with *Bifidobaterium lactis*.

Other preferred probiotics are Lactobacillus GG (ATCC 53103) and Bifidobacterium longum.

The probiotics according to the present aspect of the invention are preferably present in an amount of 10^6 to 10^9cfu/ grams of dry product, preferably 10^6 to 10^8 cfu/g, and even more preferably $2*10^7$ cfu/grams of dry product.

The composition according to the present invention comprises at least one probiotic strain. Preferably, such probiotic is a Bifidobacteria, and more preferably is *Bifidobacterium lactis*. It may also be a Streptococcus, the preferred one being *Streptococcus thermophilus*. In a very preferred embodiment of the invention, infant and starter formulae comprise *Bifidobacterium lactis* and follow-on formulae comprise both *Streptococcus thermophilus* and *Bifidobacterium lactis*.

The nutritional composition according to the first object of the present invention comprises a special blend of fats. Fat provides about half of the dietary energy and constitutes the major energy stores in the bodies of infants and young children. Presently, there is growing interest in the quality of the dietary lipid supply during infancy as a major determinant of growth, visual and neural development, and long-term health. Thus, the selection of the dietary lipid supply during early life is considered to be of great importance.

Because of the small size of their stomach and their limited tolerance towards hypertonic foods, infants require a concentrated source of energy. Of the 3 nutrients supplying energy, fat provides 9 kcal per gram, i.e. more than twice the energy present in carbohydrates or proteins. Most experts recommend that fat in infant formulae supply from 30% to 55% of the total energy.

Reconstituted at a rate of 67 kcal/100 ml, the composition according to this aspect of the present invention supplies a quantity of fat which is close to the average value found in breast milk.

Preferably, vegetable fats, and eventually a LC-PUFA oil mixture (fish oil & Mortierella alpina oil blend, for example) only are used in the manufacture of the composition. However, whey and skim milk contain naturally some traces of milk fat, explaining a very small percentage of milk fat in the formula.

Fatty acid composition of the diet determines fatty acid composition of all tissues, including storage tissues. The fat mixture in the infant formula therefore has an overall fatty acid composition as close as possible to that of human milk, in order to ensure similar membrane plasticity and same mobilization of energy in case of increased needs. The fat mixture supplies docosahexaenoic acid and arachidonic acid , in addition to essential fatty acids (linoleic and a-linolenic acids), as well as adequate quantities of the following fatty acids:

- Oleic acid (C18:1 ω9): recent data have shown that monounsaturated fatty acids (oleic acid being the main one) decrease total cholesterol and LDL-cholesterol concentrations.
- Palmitic acid (C16:0): it has been suggested that palmitic acid may be preferentially used for the synthesis of lung surfactant and thus would intervene in optimal development of respiratory function.
- Lauric and myristic acids (C12:0 and C14:0): they are medium chain saturated fatty acids, are easy to absorb, but need to be supplied at low levels. The EC Directive has proposed a maximum of 15% lauric acid and myristic acid in fat mixtures used for infant feeding. The present composition fat mixture preferably stays well below the maximal values allowed, for example between 11 and 12%.

In an aspect of this object of the invention, the fat source may also comprise an LC-PUFA oil mixture.

Human milk contains docosahexaenoic acid (DHA) and arachidonic acid (ARA) and thus breast-feeding provides infants with preformed LC-PUFAs. The DHA content of human milk varies considerably within populations and is strongly influenced by maternal diet. Globally, the DHA content of milk from mothers consuming Western

diets ranges from 0.1 to 0.4%, with a mean of 0.25%, whereas in mothers consuming non-Western diets, the DHA content of milk is greater, ranging from 0.1 to 1.4%, with a mean of 0.6%. However, amounts of 0.2 to 0.3% are generally accepted as representative. The ARA content of human milk is less influenced by the diet than DHA. Globally, the ARA content of human milk from mothers consuming Western diets ranges from 0.2 to 0.7%, with a mean of 0.45%, whereas in mothers consuming non-Western diets, the ARA content ranges from 0.4 to 1.2%, with a mean of 0.6%. Both DHA and ARA levels are influenced by the duration of lactation and tend to decrease from colostrum to transitional and mature milk.

In this aspect of the invention, the LC-PUFA is at least represented by DHA, preferably provided by a natural fish oil that supplies with a DHA/EPA (EPA: eicosapentaenoic acic) ratio > 4, similar to human milk. Together with DHA, the composition may provide a source of ARA, for example from fungal origin (Mortiellrella alpina, belonging to the American Type Culture Collection).

The composition according to the invention comprises at least one LC-PUFA. The preferred LC-PUFA is DHA, which can be the sole added LC-PUFA. In another aspect of the invention, both DHA and ARA are added into the formula.

In a preferred aspect of the present invention, the nutritional composition has a reduced level of electrolytes compared to standard infant and follow-on formulas. For example, the Na/K ratio (mmol) varies around 0.4, the (Na+K)/Cl ratio (mmol) varies around 1.8, Na+K+Cl varies around 34 and (Na+k)-Cl varies around 10.

The composition according to the present invention has a low phosphate content. Preferably, the calcium content varies between 35 and 45 mg/100 mL, the phosphorus content varies between 15 and 25 mg/mL, and the Ca/P ratio is comprised between 1.4 and 3.

The preferred amounts are indicated in table 3 below.

Table 3

	invention (mg/100mL)	cow's milk (mg/100mL)	breast milk – average values (mg/100mL)

Calcium	41	120	30
Phosphorus	21	90	15
Ca / P ratio	2	1.3	2

The formula according to the first object of the invention may also supply semi-essential nutrients which may be needed in particular conditions (e. g. taurine, nucleotides, carnitine, selenium).

Taurine is a free amino acid, which is not used to build up protein molecules. It has been shown to be involved in many physiological functions, e.g., as a trophic factor in the development of the central nervous system, maintaining the structural integrity of the membrane, regulating calcium homeostasis, as an osmolyte, a neuromodulator, and a neurotransmitter. It also conjugates with bile acids to form bile salts (essential for micelle formation and fat absorption).

Nucleotides are non protein nitrogen compounds which contain three characteristic components: a nitrogenous base, a sugar (ribose or deoxy-ribose), and one or more phosphate groups. Total nucleotide content in human milk represents 2 to 5% of the non-protein nitrogen. Cow's milk contains lower concentrations of nucleotides than human milk and its nucleotide profile differs markedly from that of human milk. Addition of nucleotides in the present infant formula follows the physiological pattern of nucleotides levels in human milk, with a predominance of easily metabolised pyrimidines over less desirable purines: addition of nucleotides to the infant formula is safe. The levels of addition are within the range allowed by the European Union Scientific Committee for Food and the European Directive.

Carnitine is a particular nitrogenous compound, which belongs to a group of food factors known as vitamin-like nutrients. It performs a crucial role in the energy supply of tissues during foetal life and in the neonatal period by facilitating the transport of long chain fatty acids into the mitochondria where beta-oxidation occurs. Fatty acids are indeed not able to pass in free form through the mitochondrial wall; the transfer into the mitochondria is governed by at least three enzymatic systems, namely carnitine – palmitoyl transferases I and II and carnitine -translocase, in which carnitine participates. Thus, carnitine is required for proper lipid oxidation and carnitine deficiency or low carnitine intake can lead to impaired fat utilisation and altered lipid metabolism. Carnitine has also a role in other metabolic processes, such as ketogenesis, lypolysis, and the maintenance of thermogenesis and nitrogen metabolism. Moreover, carnitine has been shown to improve utilisation of medium chain triglycerides in infants.

Newborns have relatively low carnitine reserves and a very low activity of the enzyme catalysing the last step in the carnitine synthesis. Thus newborns are particularly at risk of becoming carnitine-deficient in the absence of an adequate supply of exogenous carnitine. Carnitine is preferably added to infant formulae, in order to reach a level close to that of human milk.

The product according to the first object of the invention may be in powder form or as a ready to drink solution.

In the case of a powder formulae, the following feeding table may be used as a guide. However, the quantities may be changed according to medical advice. The introduction of cow's milk or any other infant formula must be carried out under medical supervision. The standard reconstitution of the formula according to the invention is 12.9%, i.e. 12.9 g powder for 90 mL of water, which gives a caloric density of 67 kcal/100mL.

Table 4

Age of infant	quantity per feed		No. of feeds per day	
	Previously boiled water (mL)	number. of measuring scoops	Formula	Others
1st and 2nd weeks	90	3	6	-
3rd and 4th weeks	120	4	5	-
2nd month	150	5	5	-
3rd and 4th months	180	6	5	-
5th and 6th months	210	7	5	-
from the 7th month onwards	210	7	4-3	1-2

In the case of a ready-to-drink solution, a special care needs to be given so that the probiotics do not enter in contact with the liquid formula accidentally. Preferably, the probiotics are stored in powder apart from the liquid formula, and is incorporated and homogenised into the liquid formula just before consumption, i.e up to two hours before consumption.

A second object of the present invention is a method for promoting physical development of an infant or a young child consisiting in fully or partly feeding said infant or child with a formula as described above, i.e. comprising proteins and probiotics, wherein at least 40% of the proteins are whey proteins comprising no CGMP or reduced CGMP.

The composition according to the invention has been shown to provide nutritional benefits including a better protein utilisation, a plasma amino acid pattern close to those of breast-fed infants, and adequate growth rates.

The improved amino acid composition according to the first object of the present invention results in better protein utilisation, as shown by the higher percentage of nitrogen retention found in infants fed with a composition according to the invention than that fed with regular whey-adapted formula (see table 5). As a Result, the total amount of total nitrogen remains unchanged.

Table 5; nitrogen balance

	whey-adapted standard formulae	formulae according to the invention
absorption	89.5%	89.3%
retention	32.2%	39.6%

Plasma amino acid in infants fed with a composition according to the first object of the invention have been shown to be closer to those of breast-fed infants compared to those fed with standard whey-adapted formulae. Furthermore, and contrary to previous studies conducted in infants fed formulae containing a protein density below 2.0g/100 kcal, plasma threonine and tryptophan levels that were critical points are now comparable to those of breast fed infants.

Furthermore, the protein content of the formula of the invention meets the needs of normal term infants during the first months of life without excessive energy intakes or increased body mass index. Still further, weight and length gains of infants fed with the formula according to the invention are comparable to breast-fed infants. (see Fig 1 to 3, and Example 1)

In Fig. 1 and 2, white columns represent a feeding with standard whey-adapted infant formulas, light grey columns a feeding with human milk, and dark-grey a feeding with the formulae according to the invention.

In Fig.3, black lozenges represent a feeding with human milk, dark grey squares a feeding with a formula according to the invention, light grey triangles a feeding with standard whey adapted formulae.

Another aspect of this object of the invention is a reduced load on immature organs. Amino acids consumed in excess and not used for protein synthesis accumulate in the blood (hyperaminoacidemias) and are metabolised in the liver into urea (i.e. the end product of unused amino acids) that needs to be excreted through the kidneys, thus

increasing kidney load. This unnecessary metabolic stress is well illustrated by plasma amino acid levels and plasma urea levels above those observed in breast-fed infants.
The lower protein content of the formula according to the invention reduces metabolic stress on infant immature organs due to excess dietary protein intake. This beneficial effect has been demonstrated: infants fed with a composition according to the invention have plasma urea nitrogen concentrations similar to those found in breast fed infants, and significantly lower than those found in the infants fed standard whey-adapted formulae. Plasma urea nitrogen is a very sensitive indicator of the adequacy of protein intake as higher levels that in breast-fed infants denote excess amino acids not utilised, whereas lower levels denote insufficient protein supply.

According to a third object of the present invention, there is provided a method for improving gastro-intestinal comfort of an infant or a young child consisiting in fully or partly feeding said infant or child with a formula as described above, i.e. comprising proteins and probiotics, wherein at least 40% of the proteins are whey proteins comprising no cGMP or reduced cGMP.
It has in particular been found a reduction of the frequency of hard stools in infants fed with a formula according to the invention.

According to the last object of the present invention, there in provided a method for developing a healthy gut microflora in an infant or a young child consisiting in fully or partly feeding said infant or child with a formula as described above, i.e. comprising proteins and probiotics, wherein at least 40% of the proteins are whey proteins comprising no cGMP or reduced cGMP.
The presence of bifidobacteria in the stools of infants fed with *B. lactis*-containing formula is similar to that observed in breastfed infants had been reported. A similar observation has been done with the formula according to the present invention. Moreover, the intestinal microflora with the formula according to the present invention follows the same pattern of evolution as in breastfed infants, whereas bifidobacteria tend to decrease when a formula identical to the formula of the invention but without *B. lactis* addition is fed to the infant.
Among anaerobic bacteria commonly found in the intestine of the new-born infant, bifidobacteria expand rapidly to become the predominant strain in the faecal microflora of breastfed infants. This is in contrast to the bottle-fed infants, whose faecal microflora hosts, besides bifidobacteria, other dominant species including enterobacteria, Enterococci and Bacteroides.

The gut microflora composition and the population size of strains is reported to be mainly regulated by competition for nutrients and oxygen availability. Although not wishing to be bound by theory, it is thought that some factors in breast milk including lactose and oligosaccharides, low iron, growth factors, nucleotides are also to be bifidogenic. Bifidobacteria, when growing, use lactose as substrate to produce lactic and acetic acids that decrease the intestinal pH to 4-5. The low buffering capacity of breast milk would allow maintenance of such a low pH, which inhibits the development of anaerobic putrefactive bacteria and enables the proliferation of Bifidobacteria that are acid-tolerant.

The addition of lactic acid bacteria to formulas, i.e., the use of probiotics, seems clinically promising: the potential for lactic acid bacteria to modify the intestinal flora and its metabolism may be beneficial in a number of conditions.

The reduced phosphate content of the formula according to the first object of the present invention optimises bone formation and, together with lactose and low protein content, creates optimal condition for an intestinal flora with a predominance of Bifidobacteria.

Examples

The following examples are illustrative of some of the products and methods of making the same falling within the scope of the present invention. They are not to be considered in any way limitative of the invention. Changes and modifications can be made with respect to the invention. That is, the skilled person will recognise many variations in these examples to cover a wide range of formulas, ingredients, processing, and mixtures to rationally adjust the naturally occurring levels of the compounds of the invention for a variety of applications.

Example 1: assessing growth

The growth is assessed by the following anthropometric measures:

Weight (nearest 10 grams): infants are weighed without clothing on electronic weighing scales. The same scales are used for all infants at all visits. The electronic weighing scales are calibrated as per the manufacturer's recommendations at the start of the study and every 3 months thereafter until the end of the study.

Recumbent length (nearest 1 mm): infants are measured using a standardised length board. At least two people are present to maintain proper body alignment and full body extension with feet flexed.

Head circumference (nearest 1 mm): obtained using a standard non-elastic, plastic coated measuring tape. The measurement is taken approximately 2.5 cm above the eyebrows, directly over the largest circumference of the skull.

Example 2 : Preferred formula

Nutrient	per 100kcal	per litre
Energy (kcal)	100	670
Protein (g)	1.83	12.3
Fat (g)	5.3	35.7
Linoleic acid (g)	0.79	5.3
α-Linolenic acid (mg)	101	675
Lactose (g)	11.2	74.7
Minerals (g)	0.37	2.5
Na (mg)	23	150
K (mg)	89	590
Cl (mg)	64	430
Ca (mg)	62	410
P (mg)	31	210
Mg (mg)	7	50
Mn (µg)	8	50
Se (µg)	2	13
Vitamin A (µg RE)	105	700
Vitamin D (µg)	1.5	10
Vitamin E (mg TE)	0.8	5.4
Vitamin K1 (µg)	8	54
Vitamin C (mg)	10	67
Vitamin B1 (mg)	0.07	0.47
Vitamin B2 (mg)	0.15	1.0
Niacin (mg)	1	6.7
Vitamin B6 (mg)	0.075	0.50
Folic acid (µg)	9	60
Pantothenic acid (mg)	0.45	3
Vitamin B12 (µg)	0.3	2
Biotin (µg)	2.2	15
Choline (mg)	10	67
Fe (mg)	1.2	8
I (µg)	15	100
Cu (mg)	0.06	0.4
Zn (mg)	0.75	5

The product will contain the additional ingredients:

Bifidobacterium Lactis (BL) : $2x10^7$ /gram of dry product

Claims

1. Infant formula comprising proteins and at least one probiotic, wherein at least 40% of the proteins are modified sweet whey proteins comprising no CGMP or reduced CGMP.

2. Infant formula according to claim 1 wherein at least 60% of the proteins are modified sweet whey proteins comprising no CGMP or reduced CGMP.

3. Infant formula according to claim 1 or claim 2 wherein the infant formula is a follow-on formula.

4. Infant formula according to one of claims 1 to 3, wherein at least one of the probiotics is a Bifidobacteria or a Lactobacillus, preferably *Bifidobacterium lactis*.

5. Infant formula according to one of claims 1 to 4 wherein at least one of the probiotics is *Streptococcus thermophilus*.

6. Infant formula according to one of claims 1 to 5 wherein the proteins are non-hydrolysed proteins.

7. Infant formula according to one of claims 1 to 6 wherein the proteins are present in a maximum proportion of 2g/100 kcal, preferably 1.85, most preferably between 1.8 and 1.85 g/100 kcal.

8. Infant formula according to one of claims 1 to 7 wherein the whey proteins with reduced CGMP represent at least 60% of the total proteins, preferably at least 70% of the total proteins.

9. Infant formula according to one of claims 1 to 8 further comprising at least one LC-PUFA.

10. Infant formula according to claim 9 wherein the LC-PUFA comprises DHA, associated or not with ARA.

11. Infant formula according to one of claims 1 to 10 further having a low amount of electrolytes.

12. Method for promoting physical development of an infant or a young child consisiting in fully or partly feeding said infant or child with a formula comprising proteins and probiotics, wherein at least 40% of the proteins are whey proteins comprising no cGMP or reduced cGMP.

13. Method for promoting physical development according to claim 12 with a formula acording to any of claims 1 to 11.

14. Method according to claim 12 or claim 13 wherein the physical development of the infant or child is characterised by a reduced load on immature organs.

15. Method for improving gastro-intestinal comfort of an infant or a young child consisiting in fully or partly feeding said infant or child with a formula comprising proteins and probiotics, wherein at least 40% of the proteins are whey proteins comprising no cGMP or reduced cGMP.

16. Method for improving gastro-intestinal comfort according to claim 15 with a formula acording to any of claims 1 to 11.

17. Method for developing a healthy gut microflora in an infant or a young child consisiting in fully or partly feeding said infant or child with a formula comprising proteins and probiotics, wherein at least 40% of the proteins are whey proteins comprising no cGMP or reduced cGMP.

18. Method for developing a healthy gut microflora according to claim 17 with a formula acording to any of claims 1 to 11.

Abstract

The formula of the invention, intended both for infants and young children, comprises proteins, preferably modified sweet whey proteins free or almost free of CGMP, and at least one probiotic.

The invention also pertains to methods for promoting physical development, method for improving gastro intestinal comfort, and method for developing a healthy gut microflora in infants or young children by fully or partly feeding them with the afore-mentionned formula.

Fig. 1

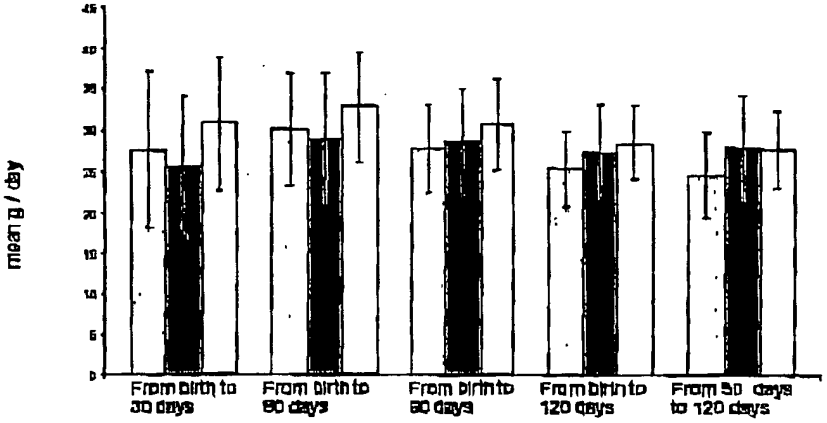

Fig. 2

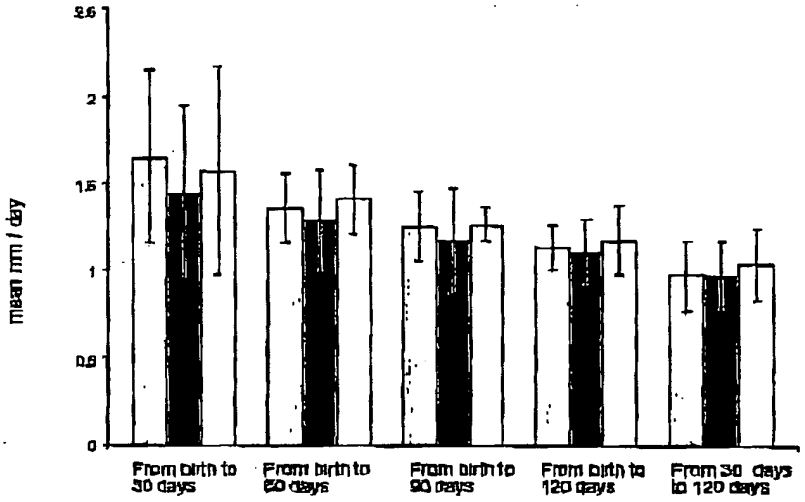

Fig. 3